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**Origin and preservation of bacteriohopanepolyolsignatures in *Sphagnum* peat from Bissendorfer Moor (Germany)**

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**Abstract**

Distributions of bacteriohopanepolyols (BHPs) were investigated in a peat core from the Bissendorfer Moor (Germany) in order to test the utility of BHPs as indicators of microbial processes in peats. Between 13 and 22 BHPs were identified in each sample (23 structures in total), with total concentrations ranging from 160 – 2800  $\mu\text{g g}^{-1}\text{TOC}$ . We have tentatively ascribed sources of most BHPs observed at this site via comparison of known BHPs source organisms with recent microbiological studies on the peat microbiome. Members of the Alpha-, Beta- and Gammaproteobacteria and specifically the genera *Burkholderia*, *Bradyrhizobium* and *Rhodoblastus*, as well as other phyla including the cyanobacteria, Acidobacteria and Acetobacteria are amongst the most likely sources. Additionally, BHP signatures which could be assigned directly to methane oxidising bacteria (35-aminobacteriohopanepentol and 35-aminobacteriohopanepentol) were

27 present only at very low levels, supporting previous studies which have shown that the  
28 majority of precursor organisms biosynthesising hopanoids in peat environments are  
29 heterotrophs. The surface layers also contained a highly unusual signature comprising  
30 high concentrations of unsaturated compounds, including unsaturated  
31 bacteriohopanetetrol pseudopentose, which has previously only been reported in  
32 *Gloeocapsa* cyanobacteria. This genus is known to occur in symbiotic association with  
33 host *Sphagnum* species, and has the ability to fix atmospheric nitrogen which is a well  
34 known trait amongst members of the peat microbiome and amongst hopanoid producing  
35 microorganisms. The apparent capacity for hopanoids to protect organisms from external  
36 stresses such as low pH is therefore likely to be a significant factor accounting for the high  
37 BHP contributions from heterotrophs, methanotrophs and phototrophic organisms in  
38 *Sphagnum* peats.

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## 1. Introduction

Peatlands contain vast stocks of organic carbon (OC) with Northern areas (including boreal and subarctic peatlands above 45° N) currently storing around 547 Gt OC as waterlogged peat (Yu et al., 2010). Carbon cycling in peats is important in terms of degradation or preservation of organic matter, with the former culminating in methanogenesis (Ciais et al., 2013). These processes are affected by changes in temperature, water table depth and organic matter content (Gorham, 1991; Kotsyurbenko et al., 2007 and references therein). New proxies for unravelling these processes in modern and ancient settings could therefore be useful for elucidating the environmental controls regulating carbon cycling and methane emissions (e.g. Pancost et al., 2011; Chambers et al., 2012; Zheng et al., 2014).

Until recently, microbiological investigations of organisms inhabiting peat bogs, including their adaptation to harsh conditions of low pH and low nutrient input, has largely focussed on organisms with specific metabolisms such as methanogenic archaea and methanotrophic bacteria as they have direct relevance to carbon cycling and climate change (see review by Andersen et al., 2013 and references therein). However, the use of visualisation techniques such as fluorescence in situ hybridisation and confocal laser scanning microscopy have revealed that *Sphagnum* mosses host a wide range of endophytic organisms in their dead hyaline cells (e.g. Opelt and Berg, 2004; Opelt et al., 2007; Bragina et al., 2012a; Shcherbakov et al., 2013). Of the organisms inhabiting the hyaline cells, methanotrophs are of particular interest as they limit the release of methane to the atmosphere and have also been shown to provide CO<sub>2</sub> to the host *Sphagnum* plants (Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010). They may also provide a significant source of fixed nitrogen which is otherwise typically limited in these systems under low atmospheric N deposition (Auman et al., 2001; Larmola et al., 2014). Studies of genes encoding nitrogenase reductase proteins (*nifH*) in *Sphagnum* mosses have

72 revealed that they are mainly derived from *Alphaproteobacteria*, a group of highly diverse  
73 organisms including phototrophs, heterotrophs and methanotrophs (Bragina et al., 2012b).  
74 However, the ability to fix N<sub>2</sub> is also widely distributed in other phyla across the *Sphagnum*  
75 peat microbiome; for example Betaproteobacteria of the genus *Burkholderia* sp., are also  
76 known to be important sources of fixed N<sub>2</sub> in peatlands (e.g. Belova et al., 2006; Opelt et  
77 al., 2007; Bragina et al., 2013; Shcherbakov et al., 2013).

78 The ability to fix nitrogen and/or oxidise methane is a very common, although not  
79 universal trait, amongst organisms which produce hopanoids (e.g. Pearson et al., 2007;  
80 Blumenberg et al., 2012; Ricci et al., 2014). Hopanoids are pentacyclic triterpenoid lipids  
81 produced by some bacteria (e.g. Rohmer et al., 1984; Ourisson et al., 1987; Ourisson and  
82 Rohmer, 1992; Farrimond et al., 1998; Talbot et al., 2008; Pearson et al., 2009). These  
83 compounds typically consist of a C<sub>30</sub> ring system (**I**; see Appendix), although variations  
84 such as methylation at C-2 (**II**) or C-3 (**III**) and unsaturation at C-6 and/or C-11 (**IV-VI**) do  
85 occur; however, the latter are rarely observed in environmental materials. They can also  
86 contain an extended polyfunctionalised side chain derived from D-ribose (Flesch and  
87 Rohmer, 1988) and are termed bacteriohopanepolyols (BHPs).

88 Typical structures have functional groups present at C-32, 33, 34 and 35, the most  
89 common example being bacteriohopane-32,33,34,35-tetrol (BHT, **Ia**). Structures with  
90 additional hydroxyl moieties at C-31 and/or C-30 are also known (e.g. Rohmer, 1993).  
91 BHPs can be broadly assigned into one of two categories “non-composite” and  
92 “composite” with the former only containing a simple functionality at the C-35 position such  
93 as -OH or -NH<sub>2</sub> including BHT (**Ia**) and 35-aminobacteriohopane-32,33,34-triol (**Id**) whilst  
94 the latter have a more complex functionality such as a sugar or aminosugar at C-35. Some  
95 structures are only known to be produced by certain groups of organisms (see summary in  
96 Table 1; Rohmer, 1993; Talbot and Farrimond, 2007; Talbot et al., 2008, 2014) providing a  
97 potentially powerful tool for profiling the microbial community, as recently demonstrated in

98 Siberian permafrost by comparison with genomic profiling (Höfle et al., 2015). Other BHPs  
99 have been related to specific environmental settings. For example adenosylhopane (**lg**) is  
100 a biosynthetic intermediate in the synthesis of other elongated hopanoids (Bradley et al.,  
101 2010), which only seems to accumulate significantly in soils (e.g. Cooke et al., 2008a; Xu  
102 et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011). These compounds have been  
103 used to trace the transport of terrestrial (soil) organic matter to marine sediments (e.g.  
104 Cooke et al., 2008b; Cooke et al., 2009; Handley et al., 2010; Taylor and Harvey, 2011;  
105 Zhu et al., 2011; Doğrul Selver et al., 2012, 2015; Wagner et al., 2014).

106 BHPs appear to perform a regulating and rigidifying function similar to that of some  
107 sterols in eukaryotes (Kannenberg and Poralla, 1999 and references therein; Sáenz et al.,  
108 2012). Although their exact function remains unclear, their regulation has been linked to a  
109 variety of environmental factors including temperature, pH, moisture limitation (e.g.  
110 Kannenberg and Poralla, 1999; Poralla et al., 2000; Joyeux et al., 2004; Welanders et al.  
111 2009; Kulkarni et al., 2013) and also growth phase (Joyeux et al., 2004; Doughty et al.,  
112 2009; Welanders and Summons, 2012).

113 Classically, investigations of bacterial communities in peat bogs using hopanoids  
114 have focussed on the geohopanoide degradation products of BHPs, such as hopanoic  
115 acids, hopanols and hopanes (e.g. Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995;  
116 Pancost et al., 2003; McClymont et al., 2008) and total hopanoide abundance has been  
117 used to study relative changes in bacterial biomass in the past (Pancost et al., 2003). In  
118 comparison, there have been few previous reports of intact polyfunctionalised BHPs in  
119 peat. BHT was first reported by Ries-Kautt and Albrecht (1989). More recently Kim et al.  
120 (2011) found between 5 and 16 BHPs in four peat samples from the catchment of the  
121 River Têt (France). Van Winden et al. (2012a) also reported the BHP composition of  
122 *Sphagnum* moss and underlying peat (to a depth of 10 cm) from a site at Moorhouse (UK),  
123 an acidic ombrotrophic blanket bog, and identified up to 13 BHP structures, including

124 markers for methane oxidising bacteria, albeit in low abundance. Similar distributions were  
125 also reported from a *Sphagnum* peat core from Belgium between 13 and 100 cm depth  
126 (van Winden et al., 2012b).

127         These earlier studies indicate the potential for BHPs to be well preserved in peat  
128 but have been limited in age/depth resolution, typically less than 400 years in age and  
129 likely within the zone of a thriving bacterial population or focused on intermediate depths  
130 without comparable surface samples (van Winden et al., 2012b). The persistence of these  
131 signatures to greater depth in peat deposits has not been investigated. Therefore, we  
132 report for the first time a full characterisation of intact BHPs in peat samples from  
133 Bissendorfer Moor (BM, Germany) to a depth of 410 cm and an age of ~2,900 cal. yr BP  
134 (Pancost et al., 2011). We focus in particular on the surface samples within the range of  
135 seasonal water table fluctuations. The primary objectives were to explore whether the  
136 diversity of BHPs reflects the predominantly heterotrophic microbially-mediated processes  
137 that occur in peat, determine whether nitrogen fixation and methane oxidation signatures  
138 could be identified against this background, and to explore how well such BHP-based  
139 signals are preserved at depth. A secondary objective was to compare peat BHP  
140 distributions to those determined for other settings; it is expected that they will be broadly  
141 similar to soil distributions, but specific characteristics of the peat environment, including  
142 nutrient limitation, a relatively low pH and strongly reducing conditions, could induce  
143 profound differences.

144

## 145 **2. Materials and methods**

### 146 *2.1. Site description and samples*

147         Peat samples were collected from Bissendorfer Moor, Germany (9.683065 E,  
148 52.506028 N; McClymont et al., 2011). This ombrotrophic bog lies 50 metres above sea-  
149 level and has been designated as a nature conservation area of 498 ha since 1971. The

150 vegetation comprises a treeless central area with mainly cotton grass and heather.  
151 Hollows and hummocks are not sharply differentiated with *Sphagnum magellanicum*, *S.*  
152 *rubellum*, and *S. papillosum* growing on hummocks and *S. cuspidatum* and *S. recurvum* in  
153 hollows (Pancost et al., 2011). Birch and pinewood dominate in the surrounding dry areas.  
154 The average annual temperature at the site is 8.9°C (range 0.6 to 17.2°C) and pH in the  
155 range 3.8 to 4.3 (Charman et al., 2007). Water table depth is dynamic, and ranged  
156 between 0 and 56 cm (measured in late summer 2003; Charman et al., 2007), with a  
157 modern depth of 20 cm indicated by a reconstruction based on testate amoebae  
158 assemblages (Charman et al., 2007; Pancost et al., 2011). Despite having conservation  
159 status, the surface hydrology has been strongly affected by drainage, meaning that the  
160 shallow subsurface microbial assemblages could have been affected by the recent human  
161 activity at this peatland (Pancost et al., 2011). The core used in this study was 422 cm  
162 long and has been analysed for macrofossils and pollen, testate amoebae and  
163 humification indices as part of the ACCROTELM project (e.g. Yeloff et al., 2006; Charman  
164 et al., 2007; McClymont et al., 2008; Pancost et al., 2011). Macrofossils in the core have  
165 been dated, and calibrated ages are based on 'wiggle matched' AMS radiocarbon dates,  
166 using software for Bayesian age-depth modelling (Pancost et al., 2011 and references  
167 therein). Samples were stored at -20°C and were freeze-dried prior to analysis.

168

## 169 2.2 Total Organic Carbon (TOC) analysis

170 Aliquots were analysed in duplicate using a Carlo-Erba EA1108 elemental analyser  
171 to determine percentage carbon, nitrogen and hydrogen. Percentage inorganic carbon was  
172 determined using a Strohlein Instruments Coulomat 702 carbon analyser adapted to  
173 analyse CO<sub>2</sub> liberated from H<sub>3</sub>PO<sub>4</sub> digestion. Elemental compositions were determined as  
174 percent of the dry weight of peat analysed. Total organic carbon was calculated as the



175 difference between total percentage carbon and total inorganic carbon (McClymont et al.,  
176 2008).

177

### 178 *2.3. Extraction and derivatisation,*

179 Lipids were extracted from approximately 0.1 - 0.3 g of freeze-dried, ground peat  
180 using repeated ultrasonication (x 3) with 5 ml of dichloromethane/methanol (1:1, v/v). This  
181 protocol was originally applied to recover and quantify neutral lipids with a focus on  
182 determining palaeoclimate information as part of the ACCROTELM project (McClymont et  
183 al., 2011, Pancost et al. 2011). Twenty two sample extracts were selected for BHP analysis  
184 via LCMS. After addition of the internal standard (5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol), aliquots of the  
185 TLE were analysed as acetates, formed by heating with acetic anhydride/pyridine (4 ml;  
186 1:1 v/v) at 50°C for 1 h and leaving at room temperature overnight. The derivatised extract  
187 was rotary evaporated to dryness and redissolved in 500  $\mu$ L MeOH/propan-2-ol (6:4 v/v)  
188 for LC-MS analysis.

189

### 190 *2.4. LC-MS analysis*

191

192 Reversed-phase HPLC was performed using a Surveyor HPLC system  
193 (ThermoFinnigan, Hemel Hempstead, UK) fitted with a Phenomenex (Macclesfield, UK)  
194 Gemini C<sub>18</sub> 5  $\mu$ m column (150 mm x 3.0 mm i.d.) and a security guard column cartridge of  
195 the same material. Separation was achieved at 30°C with a flow-rate of 0.5 mL min<sup>-1</sup> and  
196 the following gradient profile: 90% A and 10% B (0 min); 59% A, 1% B and 40% C (at 25  
197 min), then isocratic to 45 min, returning to the starting conditions in 5 min and stabilising  
198 for 10 min before injecting the next sample (A = MeOH, B = water, C = propan-2-ol; all  
199 HPLC grade from Thermo Fisher Scientific).

200 LC-MS<sup>n</sup> was performed using a Finnigan LCQ ion trap mass spectrometer equipped  
201 with an atmospheric pressure chemical ionization (APCI) source operated in positive ion  
202 mode. LC-MS settings were as follows: capillary 155°C, APCI vaporiser 490°C, corona  
203 discharge current 8  $\mu$ A, sheath gas flow 40 and auxiliary gas 10 (arbitrary units). LCQ  
204 instrument parameters were selected using an automated tune facility on a direct infusion  
205 of an acetylated standard of bacteriohopanetetrol cyclitol ether on the protonated  
206 molecular ion,  $m/z$  1002 ( $[M+H]^+$ ). LC-MS<sup>n</sup> analysis was carried out in data-dependent  
207 mode with three scan events: SCAN 1 – full mass spectrum,  $m/z$  300-1300; SCAN 2:  
208 data-dependent MS<sup>2</sup> spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent  
209 MS<sup>3</sup> spectrum of most intense ion from SCAN 2. Detection was achieved at an isolation  
210 width of  $m/z$  5.0 and fragmentation with normalised collisional dissociation energy of 35%  
211 and an activation Q value (parameter determining the  $m/z$  range of the observed fragment  
212 ions) of 0.15.

213 Structures assigned are based on comparison with authentic standards and  
214 published spectra where possible (Talbot et al., 2003a,b, 2007a,b, 2008) or by comparison  
215 of APCI MS<sup>2</sup> and MS<sup>3</sup> spectra with those of known compounds, as indicated below. The  
216 location of additional ring system methylation can be determined from relative retention  
217 times, with C-2 and C-3 methylated structures eluting ca. 0.7 and 1.3 min, respectively,  
218 after non-methylated compounds.

219 A semi-quantitative estimate of BHP abundance ( $\pm 20\%$ ) is calculated from the  
220 characteristic base peak ion peak areas of individual BHPs in mass chromatograms (from  
221 SCAN 1) relative to the  $m/z$  345 ( $[M+H-CH_3COOH]^+$ ) base peak area response of the  
222 acetylated 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\beta$ -diol internal standard. Averaged relative response factors  
223 (from a suite of five acetylated authentic BHP standards) are used to adjust the BHP peak  
224 areas relative to that of the internal standard where BHPs containing one or more nitrogen  
225 atoms give an averaged response approximately 12 times that of the standard and

compounds with no nitrogen atoms give a response approximately 8 times that of the standard (van Winden et al., 2012a).

### 3. Results

#### 3.1 TOC

TOC (%) contents in the upper section of the core (0-150 cm) are relatively stable, with a mean value of  $43.4\% \pm 1.34$  ( $\pm 1$  standard deviation; min. 40.2%, max, 45.7%,  $n = 73$ ). In the deeper section (350 – 422 cm), TOC contents are generally higher although more variable with an average of  $46.1\% \pm 2.5\%$ , (min 39.1%, max 49.1%,  $n = 31$ ).

#### 3.2 Bacterioplanepolyols (BHPs)

A total of 23 different BHPs (Tables 1 and 2) were identified in the 22 peat samples investigated, with each individual sample containing between 13 and 22 BHPs (Table 2). Total BHP abundance ranged from 150 to 2800  $\mu\text{g g}^{-1}\text{TOC}$  (Table 2). The highest concentrations of BHPs were present in the 2-4 cm and 26-28 cm layers, within the region of water table fluctuation (0- 56 cm) and also in the four deepest layers ( $> 400$  cm).

The total BHP concentration (Table 2) and those of major individual BHPs (Fig. 1, 2) show a similar depth profile, with highest values in the 2-4 cm, 26-28 cm and deepest samples ( $>400$  cm) but with consistently low-to-intermediate values throughout the rest of the profile (e.g. Fig. 1, 2). The distributions are dominated by saturated tetrafunctionalised BHPs which account for over 80% of the total BHPs in all samples from below 10 cm. Three compounds in particular comprise the bulk of the total tetrafunctionalised BHPs: BHT (**Ia**; Fig. 1a), BHT cyclitol ether (**Ij** and/or **Ik**; Fig. 1b) and aminotriol (**Id**; Fig. 1c)

accounting for over 55% of the total BHPs in all except the surface and 4-6 cm samples. Several other less abundant BHPs do not follow this general trend as discussed below.

### 3.2.1 35-amino functionalised BHPs

Aminotriol (**Id**; Fig. 1c) was the most abundant compound in 14 of the 22 samples. It always co-occurred with aminotetrol (**Ie**; Fig. 1d), although aminotriol is consistently and significantly more abundant (up to  $910 \mu\text{g g}^{-1}\text{TOC}$  compared to  $<40 \mu\text{g g}^{-1}\text{TOC}$  for aminotetrol; Table 2). The related hexafunctionalised aminopentol (**If**; Fig. 1e), was only observed below 22 cm and was the only compound identified which showed this profile. Where present, it only occurred in very low concentrations  $<5 \mu\text{g g}^{-1}\text{TOC}$  (Fig. 1e; Table 2) and never represented more than 0.5% of the total BHPs. Two minor methylated aminotriols were also observed, 2-methyl and 3-methyl-aminotriol (**IIb** and **IIIb**; Table 2).

### 3.2.2 Composite BHPs

With the exception of the surface and 4-6 cm samples, the composite structure BHT cyclitol ether (**Ij** and **Ik**; Fig. 1b) was the most abundant or 2<sup>nd</sup> most abundant compound in all samples (Table 2). The isomeric compound BHT glycoside (**Im**; Table 2) was also present in all but one sample but at significantly lower abundance, similar to the saturated bacteriohopanepentol- and bacteriohopanehexol-cyclitol ethers (**In** and **Io**, respectively; Table 2). A novel composite non-methylated hexafunctionalised BHP with a previously unrecognised terminal group structure (indicated by ion of  $m/z$  344 in  $\text{MS}^2$  spectrum of  $m/z$  1132; data not shown) was also observed in the surface samples and below 400 cm, but was only rarely observed in the upper section (0-130 cm; Table 2).

### 3.2.3 Unsaturated BHPs

276 The surface and 4-6 cm samples had unusual BHP signatures, dominated by  
277 unsaturated compounds. These compounds are also present in significant amounts in the  
278 2-4 and 8-10 cm samples (Fig. 2a,b; Table 2). A pair of peaks in the  $m/z$  653 mass  
279 chromatograms have mass spectra consistent with the previously reported spectra of  
280 monounsaturated tetrols (cf. Talbot et al., 2007b, 2008). An unsaturated aminotriol (**IVb** or  
281 **Vb** or with unsaturation in the side chain [van Winden et al., 2012a]; Table 2) was also  
282 observed, primarily in the shallower sections. Also present are two unsaturated composite  
283 BHP compounds, including an unsaturated bacteriohopanepentol cyclitol ether (**IVn** or **Vn**;  
284 Table 2). The second is a composite tetrafunctionalised BHP, previously proposed to  
285 contain a pentose terminal group (**IVi** or **Vi**, Fig. 2b; Talbot et al., 2008). The unsaturated  
286 BHT pentose is particularly dominant in the surface sample, comprising 37% of total  
287 BHPs; however, its abundance, both total and relative to other BHPs, decreases rapidly  
288 with depth down core (Fig. 2b; Table 2). The related saturated homologue (**IIi**; Fig. 2c) also  
289 had its highest relative abundance in the surface layers. However, unlike the unsaturated  
290 structure (**IVi** or **Vi**; Fig. 2b), it was present throughout the core, with highest concentration  
291 in the deepest layers (>402 cm).

292 All of the aforementioned unsaturated BHPs exhibit markedly similar depth profiles.  
293 Maximum concentrations and relative abundances occur in the three most shallow  
294 horizons and then decrease dramatically down core and are not detected throughout most  
295 of the intermediate depths down to 120 cm (Fig. 2a,b). However, in the deepest part of the  
296 core, at >400 cm, these compounds are again present, although at lower relative  
297 abundance than the most shallow horizons. This is due to an even greater increase in  
298 concentration of other compounds including BHT, Aminotriol and BHT cyclitol ether (Fig.  
299 1), but still indicates good preservation of unsaturated compounds at these depths (Fig. 2).

300

#### 301 3.2.4 Adenosylhopane and related structures

Adenosylhopane (**Ig**; Fig. 2d) is always the most abundant representative of the group containing a cyclised side chain and collectively known as “soil-marker BHPs” (Cooke et al., 2008a; Zhu et al., 2011; Doğrul Selver et al., 2012). Adenosylhopane, a related structure (“adenosylhopane Type 2”) with the same cyclised side chain but an alternative terminal group, and two methylated homologues (**Ih**; **Ilg**, **Ilh** respectively; combined sum in Fig. 2e) all exhibited similar depth profiles, being most abundant in the 2-4 and 8-10 and 26-28 cm samples (Fig. 2e; Table 2). These compounds represent up to a maximum of 16% (Fig. 3a) then fall as low as 1.7% and remain low throughout the rest of the core (Fig. 3a).

### 3.2.5 Methylated BHPs

Six different methylated BHPs were detected in the peat, five of which were methylated at the C-2 position. These compounds represent between 2 and 12 % of the total BHPs in each sample (Table 2). 2-methyl BHT (**Ila**; Table 2) is present in all samples, and 2-methylaminotriol (**Ild**; Table 2) is present in all but four of the near surface samples. The three other C-2 methylated BHPs had a much more limited occurrence, with the methylated adenosylhopane and the methylated homologue of the related compound (**Ilg**, **Ilh**; Table 2) only present in a few of the surface samples above 22 cm depth. The fifth compound, 2-methylBHT pseudopentose (**Ili**), was only detected in 3 samples below 400 cm and in very low abundance (Table 2). Finally, one C-3 methylated compound was observed, 3-methyl-aminotriol (**IIlb**); however, it was only present below 18 cm depth and always at low levels (Table 2), similar to the concentration profile of aminopentol (**If**; Fig. 1e).

## 4. Discussion

328

#### 329 4.1 Sources of BHPs in BM peat

330 Microbiological studies of *Sphagnum* and peats reveal both typical bacterial groups  
331 but also pronounced variations in distribution that appear to depend largely on abiotic  
332 factors such as pH (e.g. Bragina et al., 2012a). Particularly common organisms include  
333 Alphaproteobacteria with subordinate contributions from Beta-, Gamma- and in some  
334 cases Deltaproteobacteria (e.g. Dedysh et al., 2006; Bragina et al., 2012a, b; Serkebaeva  
335 et al., 2013). Many of these bacteria are heterotrophic and some are also capable of  
336 dinitrogen fixation. In a recent study of a peat soil targeting the *nifH* gene, one of the main  
337 components of the nitrogenase complex, a high diversity of diazotrophic bacteria were  
338 detected (Zadorina et al., 2009). These sequences also included, but were not limited to,  
339 species of Alpha-, Beta-, Gamma and Deltaproteobacteria. Other important phyla in the  
340 peat microbiome include Acidobacteria, Actinobacteria, Planctomycetes and  
341 Verrucomicrobia (Dedysh et al., 2006; Bragina et al., 2012b; Serkebaeva et al., 2013).  
342 Each of these phyla are known to include hopanoid producers (Table 1; e.g. Rohmer et al.,  
343 1984; Pearson et al., 2007), although biosynthesis by members of the proteobacteria has  
344 been studied much more extensively than most of the other, more recently described phyla  
345 (Rohmer et al., 1984; Ourisson et al., 1987; Farrimond et al., 1998; Kuchta et al., 1998;  
346 Talbot et al., 2008; see also references in Table 1).

347

##### 348 4.1.1 Heterotrophs

349 As BHP abundances are high in the shallowest layers (Table 2; Figs 1-3) it is  
350 likely that many of the most abundant BHPs derive from aerobic heterotrophs. These  
351 organisms are important members of the bacterial community found in peat, consuming a  
352 wide range of organic substrates, including organic acids, sugars, polyalcohols and some  
353 aromatic compounds as carbon and energy sources (e.g. Belova et al., 2006). This is

354 consistent with previous studies on peats which suggest that  $^{13}\text{C}$ -enriched hopanes are an  
355 indicator of a heterotrophic bacterial population consuming  $^{13}\text{C}$ -enriched carbohydrates  
356 (Pancost et al., 2000; Xie et al., 2004).

357 The BHP profile of BM is broadly consistent with a dominantly heterotrophic  
358 bacterial community. Most heterotrophs make BHT (**Ia**), and the majority also make  
359 composite BHPs, with BHT cyclitol ether (**Ij** and/or **Ik**) the most commonly-occurring  
360 structure found in members of the Alpha-, Beta-, Gamma and Deltaproteobacteria (see  
361 Table 1). Members of the Betaproteobacterial genus *Burkholderia*, which comprises gram-  
362 negative, aerobic and microaerophilic chemoorganotrophic bacteria, have been shown to  
363 be particularly important and abundant in the peat microbiome (Opelt and Berg, 2004;  
364 Belova et al., 2006; Opelt et al., 2007; Sun et al., 2014) and are likely to be important  
365 sources of both BHT and cyclitol ether compounds (Table 1). A high diversity of  
366 *Burkholderia* species was found in both the endophytic and ectophytic habitats of different  
367 *Sphagnum* moss species and in relation to two species (*S. magellanicum*, Opelt et al.,  
368 2007; *S. rubellum*, Opelt and Berg, 2004) that are also present at BM.

369 The *Acetobacter* and *Gluconacetobacter* genera are known as acetic acid bacteria  
370 (AAB). They are diazotrophic (e.g. Bragina et al., 2012b) and also produce acetate which  
371 is a necessary substrate for acetoclastic methanogenesis which is an important pathway in  
372 some shallow peats (Kelley et al., 1992; Popp and Chanton, 1999; Chasar et al., 2000;  
373 Metje and Frenzel, 2005). Alphaproteobacteria AAB are prolific sources of a wide range of  
374 BHPs with core structures of BHT (**Ia**), BHT cyclitol ether (**Ij**) and BHpentol cyclitol ether  
375 (**In**) but also including mono and diunsaturated compounds with double bonds at C-6 (**IV**),  
376 C-11(**V**) or both (**VI**) and with ring systems both with and without additional methylation at  
377 C-3 (**III**; Rohmer and Ourisson, 1986; Peiseler and Rohmer, 1992; Talbot et al., 2007b).  
378 Whilst AAB are likely present in BM and a possible source for the numerous unsaturated



379 BHPs, the absence of any C-3 methylated homologues, either of the tetrol or tetra- or  
380 pentafunctionalised cyclitol ethers, suggests contributions from these organisms is minor.

381 Of the species of Deltaproteobacteria investigated, only *Geobacter* species are  
382 considered potential sources here (Table 1); however, the absence of guanidine  
383 substituted BHT cyclitol ether which co-occurs with BHT, BHT cyclitol ether and BHT  
384 glucosamine in *Geobacter* spp. suggests a contribution from these organisms is unlikely or  
385 limited at this site. Other genomic studies have shown that *sqhC* diversity related to as yet  
386 unknown Deltaproteobacteria is high, especially in soils relative to marine sediments  
387 (Pearson et al., 2009), such that contributions from other Deltaproteobacteria cannot be  
388 excluded.

389 Amongst the heterotrophs, relatively few species make only non-composite BHPs.  
390 The Alphaproteobacterial genera *Beijerinckia* and *Bradyrhizobium* contain relatively simple  
391 BHP distributions dominated by aminotriol (**Id**), with additional BHT (**Ia**) in *Beijerinckia*  
392 *indica* (Table 1). Members of the Actinobacteria also only make these less complex  
393 compounds (Table 1). However, as these compounds represent two of the three most  
394 abundant compounds at BM (Fig. 1; Table 2) contributions from such sources could be  
395 important. Unlike any of the other heterotrophs with known BHP compositions,  
396 *Bradyrhizobium japonicum* also accumulates adenosylhopane (**Ig**; Table 1), which is  
397 present in the highest concentration in the 2-4 cm sample and constituted the greatest  
398 relative proportion of the total BHPs (13.4%; Table 2) in the 10-12 cm sample, likely  
399 indicating an aerobic source such as *B. japonicum*. Adenosylhopane is a biosynthetic  
400 intermediate in the synthesis of other side chain elongated BHPs (Bradley et al., 2010);  
401 therefore, there are potentially numerous sources for this compound, although, with the  
402 exception of *B. japonicum* few organisms have been shown to accumulate it in detectable  
403 amounts (Table 1).

404 To summarise, proteobacteria are undoubtedly significant sources of BHPs at BM,  
405 with *Burkholderia* spp. considered particularly important sources of BHT (**Ia**) and BHT  
406 cyclitol (**Ij** and/or **Ik**) although multiple sources are expected for both compounds (see  
407 Table 1). *Beijerinckia* spp. are likely sources of aminotriol (**Id**) with additional contributions  
408 from *Bradyrhizobium* sp. and Actinobacteria. *B. Japonicum* likely also contributes to  
409 adenosylhopane (**Ig**).

410

#### 411 4.1.2 Aerobic methane oxidising bacteria (methanotrophs)

412 The northern peatlands cover an area of approximately 4 million km<sup>2</sup> (>45°N; Yu et  
413 al., 2010 and references therein), and are a major source of methane release to the  
414 atmosphere (e.g. Spahni et al., 2011 and references therein). Recent estimates vary but  
415 indicate the flux of methane from northern peatlands is in the range 24 to 58 Tg CH<sub>4</sub> yr<sup>-1</sup>  
416 (Zhang et al., 2016 and references therein). This release is attenuated by methane  
417 consuming bacteria (methanotrophs) inhabiting the oxic layers of the peat (e.g. Segers,  
418 1998; Dedysh, 2009) and within *Sphagnum* (Raghoebarsing et al., 2005). Culturable  
419 methanotrophs are classified phylogenetically as either members of the  
420 Alphaproteobacteria (known as Type II) or Gammaproteobacteria (known as Type I;  
421 Hanson and Hanson, 1996), with a third group recently described from the phylum  
422 Verrucomicrobia (Op Den Camp et al., 2009).

423 Dedysh (2009) described the methanotroph diversity in acidic northern wetlands  
424 and found that these settings are mainly colonized by methanotrophic representatives of  
425 the Alphaproteobacteria (i.e. Type II methanotrophs). However, studies using functional  
426 gene analysis by microarray or ultra-deep pyrosequencing of *pmoA* genes on *Sphagnum*  
427 peat samples from a range of environments including but not limited to northern regions  
428 (Siberia, Sweden, Canada, Argentina and The Netherlands), revealed Type I organisms  
429 including the genera *Methylomonas*, *Methylobacter*, *Methylomicrobium* and

430 *Methylocaldum* (Kip et al., 2010, 2011). Type II organisms related to *Methylocystis* and  
431 *Methylosinus* spp. (family Methylocystaceae) were also significant members of the  
432 methanotroph community at all sites (Kip et al., 2010, 2011) and were particularly  
433 dominant in *Sphagnum magellanicum* dominated habitats from Patagonia (Kip et al.,  
434 2012). Whilst *Methylocystis* spp. are a common and abundant group in acidic peats, there  
435 are other more recently described genera of Type II methanotrophs from the family  
436 *Beijerinckiaceae* including *Methylocella* and *Methylocapsa*, which are also important and  
437 would not have been observed in some earlier studies utilising the functional gene *pmoA*  
438 (which is not present in these organisms; e.g. Rahman et al., 2011 and references  
439 therein). Recent studies have also suggested an apparently symbiotic relationship  
440 between methanotrophs and *Sphagnum* moss (Kip et al., 2010), with a bacterium  
441 occurring inside cells of *S. cuspidatum* showing 93% 16S rRNA sequence similarity to  
442 cultured *Methylocella* and *Methylocapsa* sp. (Raghoebarsing et al., 2005).

443 Previous studies of methane oxidising bacteria have shown that they produce  
444 characteristic non-composite BHP distributions, with most Type I species producing high  
445 levels of the hexafunctionalised compound aminopentol (**If**) and lower amounts of the  
446 related pentafunctionalised compound aminotetrol (**Ie**; Table 1; e.g. Talbot et al., 2001; van  
447 Winden et al., 2012a; Talbot et al., 2014 and references therein). Some Type I organisms  
448 of the genera *Methylococcus* and *Methylocaldum* also produce homologues of  
449 aminopentol and aminotetrol with a methyl group at position C-3 (**IIIIf** and **IIIe** respectively;  
450 Neunlist and Rohmer, 1985a; Cvejic et al., 2000a), although the 3-methyl-aminotriol (**IIIb**)  
451 observed here (Table 2) has only recently been reported from cultures of the Type I  
452 organism *Methyломicrobium alcaliphilum* (Banta et al., 2015), an alkaliphilic organism  
453 unlikely to be present in *Sphagnum* peat. Type II organisms, including representatives of  
454 the genera *Methylocystis* and *Methylosinus* (Alphaproteobacteria), produce a  
455 combination of BHT, aminotriol and aminotetrol (Rohmer et al., 1984; Neunlist and

456 Rohmer, 1985b; Cvejic et al., 2000a; Talbot et al., 2001; van Winden et al., 2012a). It  
457 should be noted, however, that there are multiple other sources of aminotriol especially  
458 amongst other Alphaproteobacteria (Table 1; e.g. Talbot and Farrimond, 2007 and  
459 references therein).

460 Of the other Type II methanotrophs from the family Beijerinckiaceae, no cultured  
461 representatives of *Methylocapsa* sp. (e.g. Dedysh et al., 2001a) have been tested for BHP  
462 production or composition, but van Winden et al. (2012a) reported the BHP composition of  
463 *Methylocella palustris*. *Methylocella* and *Methylocapsa* are closely related to another  
464 known BHP producer *Beijerinckia* sp., which makes aminotriol (**Id**) and in some cases BHT  
465 (**Ia**; Table 1; Vilcheze et al., 1994) but they were not found to produce aminotetrol (**Ie**). The  
466 dominant compounds in *M. palustris* (and also *Methylocella tundrae*; Talbot and Rohmer,  
467 unpublished data) were aminotriol and BHT with trace levels of adenosylhopane (**Ig**).  
468 Unfortunately, there are numerous different sources for all of these compounds so there is  
469 no way to conclusively identify BHP contributions from *Methylocella* spp. using BHP  
470 analysis (Table 1). Given the importance of this group as demonstrated by microbiological  
471 studies (Kip et al., 2010, 2011; Rahman et al., 2011), we consider it likely that Type II  
472 methanotrophs of the Beijerinckiaceae family will be important sources of (at least)  
473 aminotriol (**Id**) at this site.

474 Although a methanotroph biomarker might be expected to be most abundant at the  
475 redox interface i.e. water table depth, where oxygen is present and methane  
476 concentrations are the highest (e.g. Sundh et al., 1995), aminotriol was present at high  
477 concentration in all samples from BM (Fig. 1c). Aminotetrol (**Ie**) was also present in all  
478 samples, representing up to 2.3% of the total BHP distribution with the highest  
479 concentration in the 26-28 cm sample (Fig. 1d). Although this is around the approximate  
480 average depth of the oxic-anoxic interface, this region of the core will be exposed to  
481 oxygen seasonally as the water table lowers. The highest concentration of aminopentol (**If**)

482 for the entire profile occurred in the same sample (Table 2), and it was only present below  
483 22 cm; however, it never represented more than 0.4% of the total BHP distribution (Fig.  
484 1e). The occurrence of aminotriol and aminotetrol together, in the absence of aminopentol,  
485 in the upper section of the core (0 to 22cm) suggests the presence of Type II  
486 methanotrophs *Methylocystis/Methylosinus* (family Methylocystaceae; Table 1) at BM  
487 which is consistent with previous studies on methanotroph populations in peat (e.g.  
488 Dedysh et al., 2001b; Dedysh, 2009; Kip et al., 2011).

489 Aminopentol (**If**), which is only known to occur in Type I methane oxidising bacteria  
490 (see review in Talbot et al., 2014), was only observed below 22 cm depth (Fig. 1e).  
491 Although this depth is within the reported range of water table variations (0-56 cm;  
492 Charman et al., 2007), it is below the current reconstructed water table level (~20 cm)  
493 based on testate amoebae analysis (Pancost et al., 2011 after Charman et al., 2007). This  
494 suggests that the occurrence of aminopentol in deeper samples likely reflects the  
495 presence of relict BHPs initially formed at the anoxic-oxic interface. As such, aminopentol  
496 is most likely a fossil compound recording past methanotrophy. The absence of C-3  
497 methylated homologues of aminopentol and aminotetrol suggest a likely source organism  
498 would be *Methylomonas* sp., consistent with the identification of this genera in *Sphagnum*  
499 peat in other studies (Kip et al., 2010) and the recent isolation of the first acid tolerant  
500 *Methylomonas* sp. from and acidic *Sphagnum* peat bog (Danilova et al., 2013).

501 We speculate that the only C-3 methylated BHP observed at BM, 3-  
502 methylaminotriol (**IIId**; Table 2), present from 18 cm depth and below is most likely related  
503 to a genera of Type I methanotrophs based on the similar depth profile to that of  
504 aminopentol (which occurs from 22 cm and below; Fig. 1a; Table 2).

505 Aminotriol concentrations are over two orders of magnitude greater than aminotetrol  
506 concentrations, inconsistent with their relative abundances in previously cultured type II  
507 methanotrophs (Neunlist and Rohmer, 1985b; Jahnke et al., 1999; Talbot et al., 2001; van

508 Winden et al., 2012a). Instead, the marked similarity of the aminotriol BHP depth profile  
509 with most other major BHPs (Fig. 1) suggests a significant non-methanotrophic origin for  
510 this compound, or a methanotroph origin from one of these more recently described  
511 sources (e.g. *Methylocella*; van Winden et al., 2012a). The similar depth profile of  
512 aminotetrol, although far less abundant (Fig. 1c and d), suggests that this might also  
513 derive, at least partially, from other sources, although the only known non-methanotroph  
514 source are sulphate reducing bacteria of the genus *Desulfovibrio* (Blumenberg al., 2006,  
515 2009b, 2012), which is unlikely to occur in this environment. Only aminopentol has a depth  
516 profile consistent with being derived predominantly from methanotrophs.

517 Finally, although anaerobic methane-oxidisers related to the novel bacterium  
518 “*Candidatus Methyloirabillia oxyfera*” have been identified in peat (e.g. Zhu et al., 2012),  
519 the diagnostic BHP produced by these organisms (3-Methyl-bacteriohopanehexol, **III**f;  
520 Kool et al., 2014) was not detected.

521

#### 522 4.1.3 Phototrophic bacteria

523 The surface layers of peat bogs host a wide range of phototrophic eukaryotes  
524 and prokaryotes. This includes nitrogen fixing cyanobacteria which inhabit *Sphagnum* peat  
525 bogs, occurring in both epiphytic and intracellular associations with *Sphagnum*, (e.g.  
526 Granhall and Selander, 1973; Krivograd Klemenčič and Vrhovška, 2003; Krivograd  
527 Klemenčič et al., 2010; Bragina et al., 2012b). Many different classes of hopanoid  
528 producing cyanobacteria, including *Anabaena*, *Calothrix*, *Cyanothece*, *Gloeocapsa*,  
529 *Microcystis*, *Nostoc*, *Oscillatoria* and *Phormidium* (Talbot et al., 2008 and references  
530 therein), have been observed in peat (Krivograd Klemenčič et al., 2010 and references  
531 therein). Until recently, cyanobacteria were considered to be the major source of  
532 hopanoids methylated at the C-2 position (Summons et al., 1999); however, recent  
533 genomic studies have revealed that the capacity for this synthesis also occurs in other

534 phyla, particularly the Alphaproteobacteria and also Acidobacteria (Welanders et al., 2010)  
535 and that it is a particularly common trait in organisms found in close (symbiotic)  
536 association with plants (Ricci et al., 2014).

537         Of particular interest to this study are the species of *Gloeocapsa* cyanobacteria  
538 which were identified, for example, in the Männikjärve bog (central Estonia) and were  
539 particularly abundant on *S. magellanicum* plants (Karofeld and Toom, 1999), one of the  
540 species present at the BM site. A further seven *Gloeocapsa* spp. were identified in two  
541 Slovenian bogs (Krivograd Klemenčič and Vrhovška, 2003). Crucially, a *Gloeocapsa* sp. is  
542 the only known source of the unsaturated compound, identified as a “BHT pentose” (**IVi** or  
543 **Vi**), that is highly abundant (over 40%) in the surface layers at the BM site (Fig. 1b). Such  
544 a high abundance in surface layers is consistent with an aerobic and/or phototrophic  
545 source and suggests a particular ecophysiological role, potentially regulating osmotic  
546 pressure or proton gradients in the low pH peat environment (cf. Welanders et al., 2009).  
547 The original identification of this compound, together with its saturated and C-2 methylated  
548 homologues (**IIIi**) was from a sample of an epilithic colony of *Gloeocapsa* sp. from Devon  
549 Island (Arctic; Talbot et al., 2008). The saturated pentose compound, with the non-  
550 methylated structure (**IIi**, Table 2, Fig. 2c), was also observed in BM peat, being present at  
551 all depths but showing a markedly different depth profile to the unsaturated structure (Fig.  
552 2b). The methylated pentose structure (**IIIi**; Table 2) was only observed in the deepest  
553 levels, below 400 cm. Therefore, the saturated compounds could: (i) derive from an  
554 additional source to the unsaturated compounds; (ii) have a markedly different  
555 preservation potential for the methylated vs non-methylated compounds; and/or (iii) have  
556 different extraction efficiencies in the fresher, near surface material, given that some other  
557 methylated BHPs have exhibited resistance to extraction (Herrmann et al., 1996; Allen et  
558 al., 2010).

559 The pair of unsaturated bacteriohopanetetrols (**IVa, b** or **c**; Table 2) also had very  
560 similar depth profiles to the unsaturated BHT-carbopseudopentose suggesting a common  
561 source (Fig. 2a and b). A single unsaturated BHT with an identical mass spectrum was  
562 also observed in the original source of the *Gloeocapsa* enrichment (Talbot et al., 2008).  
563 One possibility, therefore, is that a *Gloeocapsa* sp. inhabiting this environment produces  
564 both the composite carbopseudopentose and one or both of the observed unsaturated  
565 tetrols. An alternative heterotrophic source for the unsaturated tetrols, the  
566 Alphaproteobacteria AAB, are discussed above but considered minor at most, again due  
567 to the absence of C-3 methylated homologues.

568 Purple non-sulfur bacteria (PNSB) are normally anoxygenic photoheterotrophic  
569 organisms. They belong to the Alphaproteobacteria and Betaproteobacteria with many  
570 representatives being closely related to non-phototrophic, strictly chemotrophic bacteria  
571 (Kulichevskaya et al., 2006 and references therein). PNSB are widely distributed in various  
572 aquatic ecosystems as well as in sediments, moist soils and natural wetlands  
573 (Kulichevskaya et al., 2006), but peat bogs are rarely reported as sources of PNSB  
574 (Kulichevskaya et al., 2006). Few PNSB can tolerate low pH with the exception of  
575 members of the genera *Rhodoblastus* (Pfennig, 1969; Imhoff, 2001). Several species of  
576 *Rhodoblastus* have been isolated from acidic *Sphagnum* bogs (Kulichevskaya et al.,  
577 2006), and *Rhodoblastus acidophilus* (formerly *Rhodopseudomonas acidophila*; Imhoff  
578 2001) has been shown to produce a number of common hopanoids including BHT (**Ia**) and  
579 BHT cyclitol ether (**Ij**; Talbot et al., 2007a and references therein). The major product,  
580 however, was adenosylhopane (**Ig**, Table 1; also seen in other PNSB including  
581 *Rhodopseudomonas*; Talbot et al., 2007a and references therein; Eickhoff et al., 2013a)  
582 suggesting another possible source of adenosylhopane at BM.

583

#### 584 **4.2 Comparison of BHP distributions at BM to other soils and peat**



585 Biohopanoids were present at high concentration throughout the BM peat core (Table 2). A  
586 total of 23 different intact BHP structures were observed (Table 2), comparable to recent  
587 reports of BHP distributions in soils (e.g. Cooke et al., 2008a; Xu et al., 2009; Zhu et al.,  
588 2011; Spencer-Jones et al., 2015) and considerably more complex than typical  
589 distributions in aquatic sediments (e.g. Blumenberg et al, 2006, 2009a, 2010; Talbot and  
590 Farrimond, 2007; Coolen et al., 2008; Cooke et al., 2008b, 2009), with the exception of  
591 sediments occurring on deep-sea fans receiving high terrigenous inputs (Handley et al.,  
592 2010; Wagner et al., 2014). Although the extraction method used here (sonication in  
593 DCM:MeOH) was not the typical modified Bligh and Dyer method (e.g. Cooke et al.,  
594 2008a), which has been widely applied to BHP extractions in other studies, the BHP  
595 distributions are comparable and the concentrations in many cases are similar or higher  
596 than those reported for other peats (Kim et al., 2011; van Winden et al., 2012a,b). The  
597 highest total BHP concentrations were found in the 2-4 cm sample ( $2700 \mu\text{g g}^{-1}\text{TOC}$ ) and  
598 below 400 cm ( $1100\text{-}3000 \mu\text{g g}^{-1}\text{TOC}$ ), whilst values throughout the remainder of the core  
599 were lower, ranging from  $160 \mu\text{g g}^{-1}\text{TOC}$  (32-34 cm) to  $900 \mu\text{g g}^{-1}\text{TOC}$  (Table 2).

600 Although to date BHPs in peat have only been studied by LCMS from 3 other  
601 locations (Kim et al., 2011; van Winden et al., 2012a,b), our data collectively suggest that  
602 whilst soil and peat derived organic matter generally contain the same BHPs, they have  
603 characteristically different relative distributions, as recently suggested for tropical and non-  
604 tropical soils (Spencer-Jones et al., 2015). Excluding the surface layers, the three most  
605 dominant BHPs in the BM peat at all other depths were BHT (**la**), BHT-cyclitol ether (**lj**)  
606 and aminotriol (**ld**). Again, these observations are consistent with other recent studies of  
607 BHPs in peat (Kim et al., 2011; van Winden et al., 2012a,b) and also with the BHP  
608 composition in soils from the Northern hemisphere (Cooke et al., 2008a; Xu et al., 2009;  
609 Cooke, 2010; Rethemeyer et al., 2010; Kim et al., 2011) and tropical settings (Wagner et  
610 al., 2014; Spencer-Jones et al., 2015). However, we find that the BHP distribution at BM,

611 and other peats, differs from soils in two fundamental ways: (i) they contain a particularly  
612 high proportion of unsaturated BHPs in peat surface layers; and (ii) generally exhibit lower  
613 proportions of the 'soil-marker' BHPs.

614 The surface layers of the BM peat core contain exceptionally high concentrations of  
615 unsaturated biohopanoids, including unsaturated BHT-pentose (**IVi or Vi**), in agreement  
616 with observations by van Winden et al. (2012a) for a UK peat bog. The BM site also  
617 uniquely contained two isomers of unsaturated BHT (possibly **IVa** and/or **b** or **c**), whilst  
618 only one isomer was found in the UK study (van Winden et al., 2012a). These compounds,  
619 together with 2 other minor unsaturated BHPs (**IVd or Vd** and **IVm or Vm**) accounted for  
620 up to 46 % in the surface layers, rapidly falling to 3% or less below 12 cm depth (although  
621 all unsaturated BHPs were observed again below 400 cm depth; Fig. 2 a and b; Table 2).

622 Among the defining features of soil BHP distributions are high levels of  
623 adenosylhopane (**Ig**), its C-2 methylated homologue (**Ilg**; Talbot et al., 2007a) and two  
624 pairs of related compounds with as yet unidentified terminal groups (including  
625 "adenosylhopane type 2"; **Ih** and **IIh**, Table 1), together comprising 28% of the average  
626 total BHP assemblage (Cooke et al., 2008a; Cooke, 2010). Five soils from Canada and  
627 permafrost soils from Svalbard and Siberia contained even higher proportions of this BHP  
628 group (Xu et al., 2009; Rethemeyer et al., 2010; Höfle et al., 2015). In the peat samples  
629 studied here, the distribution of these compounds is rather different, with adenosylhopane  
630 and related compounds only present in significant concentration in the shallow sub-surface  
631 (up to 15% in 2-4 and 8-12 cm; Fig. 3a) but are otherwise below 10%. In *Sphagnum* peats  
632 from 2 other European sites (UK, Belgium; van Winden et al., 2012a and b respectively),  
633 the relative abundance of soil marker BHPs is also low, typically less than 10% except in  
634 the near surface samples (up to 15%; Fig. 3a). The only exceptions are 4 peat samples  
635 from France which have a slightly higher relative abundance of soil biomarkers (20-30%;

636 Fig. 3b), however, no details were provided as to the type of peat in that study so it is  
637 possible they were not *Sphagnum* peats (Kim et al., 2011).

638         The low values of soil marker BHPs (typically < 10%; Fig 3a,b) within peats are  
639 distinctive from those of soils. Values reported recently for tropical and sub-tropical soils  
640 had values in the range 0-40% although the majority were in the range 10-20%, slightly  
641 higher than the peat samples (Fig. 3c). Temperate soils from Canada (Alberta), France  
642 and the UK, had a wide range (0-70%; Fig. 3d) with the widest range found in permafrost  
643 soils from the Siberian Arctic and Svalbard (0-90%; Fig. 3e), possibly reflecting competing  
644 influences of low temperature leading to higher values and low pH leading to low values  
645 (Höfle et al., 2015; Spencer-Jones et al., 2015). As discussed above, the most likely  
646 sources of adenosylhopane (**lg**) and related structures in *Sphagnum* peat environments  
647 are nitrogen-fixing Alphaproteobacteria (*Bradyrhizobium* sp.; PNSB; *Methylocella* sp.;  
648 Table 1). As adenosylhopane is in itself an intermediate in the biosynthetic pathway to  
649 other side chain elongated BHPs (Bradley et al., 2010), this suggests that under the low  
650 pH conditions found in *Sphagnum* peat (pH 3.8 – 4.3 at BM; Charman et al., 2007), the  
651 species that would normally accumulate adenosylhopane convert this precursor to other  
652 BHPs. Alternatively, other nitrogen fixers such as *Burkholderia* sp. (Betaproteobacteria)  
653 and other, especially Alphaproteobacterial, BHP producers (e.g. Zadorina et al., 2009)  
654 outcompete the adenosylhopane accumulators in these systems. In their study of BHPs in  
655 Siberian permafrost, Höfle et al. (2015) reported that concentrations of adenosylhopane  
656 (and related compounds) were negatively correlated with pH, the implication being that  
657 further modification of the side chain is more important at lower pH than more neutral  
658 conditions, hence the greater diversity of structures present in low pH peat and peaty soils  
659 (Cooke et al., 2008a; van Winden et al., 2012a). Indeed Gong et al. (2015) recently  
660 reported that pH was a key factor controlling the geographical distribution of squalene  
661 hopene cyclase (*sqhC*) with Proteobacterial and Acidobacteria the dominant source

organisms in the acidic Dajiuhu peatland (China). Regardless of the reasons for this difference in BHP distributions, this variability could prove useful for tracing the origin and transport of terrestrially derived organic matter in the aquatic realm (Cooke et al., 2008b, 2009; Zhu et al., 2011; Doğrul Selver et al., 2012). Furthermore, these effects would almost certainly lead to variations in the values of the  $R_{\text{soil}}$  and  $R'_{\text{soil}}$  proxies with values at BM significantly lower than those reported recently for other soils (Table 2; see review in Spencer-Jones et al., 2015).

#### 4.3 Preservation of hopanoids at BM

This is the first study to investigate the BHP distribution in peat samples to a depth of 410 cm, equivalent to an age of ~3000 cal. yr BP. We observe robust preservation of complex, highly functionalised BHPs at BM although there are reports of similarly complex hopanoids in marine sediments from the Congo deep sea fan dating to over 2.5 Ma (Handley et al., 2010; Talbot et al., 2014; Spencer-Jones, 2016) and the oldest confirmed biohopanoid is BHT, in ~50 Ma sediments from Tanzania (van Dongen et al., 2006).

Earlier studies on lignites, peats and soils have reported the rapid conversion of biohopanoids to geohopanoids (e.g. van Dorselaer et al., 1975; Quirk et al., 1984; Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995). That is also true here, with a significant increase in geohopanoid concentrations with depth (data not shown). Nonetheless, at BM we continue to see a full suite of  $17\beta,21\beta(H)$  tetra-, penta- and hexafunctionalised BHPs even at >400 cm depth indicating favourable preservation conditions (Table 2). Intriguingly, the concentration of BHPs in the deepest samples (402-410 cm) are equivalent to those in the most concentrated near surface samples (2-4 cm; Table 2).

Although originally ascribed to a purely aerobic source, recent work has shown that there are also a number of potential obligate and facultative anaerobic sources that can produce BHPs, including Planctomycetes (Sinninghe Damsté et al., 2004; Rush et al.,

2014), *Desulfovibrio* sp. (Deltaproteobacteria; Blumenberg et al., 2012),  
*Rhodopseudomonas palustris* (Alphaproteobacteria; Rashby et al., 2007) and *Geobacter*  
sp. (Deltaproteobacteria; Fischer et al., 2005; Eickhoff et al., 2013b). The BHPs produced  
by members of these phyla and/or species are discussed above (see also Table 1);  
however, at this time few species from these groups can be directly related to peat  
environments. Therefore, although we cannot rule out some contribution from anaerobes  
at depth in the peat core, we propose that the major proportion of the BHPs are produced  
by aerobes at or above the water table and are subsequently preserved at depth.

## 5. Conclusions

A peat core from Bissendorfer Moor (Germany) contained a wide range of  
structurally distinct BHPs at high concentrations to a depth of 410 cm (c. 2900 cal. yr BP).  
By comparison with literature on *Sphagnum* peat microbiological communities, these lipids  
can be linked primarily to heterotrophic but also methanotrophic and phototrophic  
members of the peat microbiome. One of the most striking conclusions of this work, but  
one that is consistent with previous work, was the relatively small impact of bacterial  
methanotrophs on the BHP signature. Aminopentol, a biomarker for Type I methanotrophs,  
was only present below 22 cm but never represented more than 0.4% of the total BHP  
pool. Similarly aminotetrol, produced by both Type I and II methanotrophs, only accounted  
for up to 2% of total BHPs. This suggests that even in peat deposits, methanotrophs  
represent a relatively minor component of the bacterial (or at least the hopanoid-  
producing) population; settings where much higher proportions occur (e.g. Talbot et al.,  
2016) must be characterised by a particularly strong methane cycle. Collectively, the  
types of compounds present are similar to those reported from soils whilst the relative  
distributions show several distinct differences. The near surface samples contained

714 exceptionally high levels of a number of unsaturated BHPs including two isomers of  
715 unsaturated BHT, a feature which has not been reported elsewhere. A second major  
716 difference between BHPs in BM peat and those reported for soils was the relative  
717 abundance of adenosylhopane and related structures, which was as high as 15% in a few  
718 (near) surface horizons but generally below 10% and much lower (~1.7%) in deeper  
719 layers. These values are significantly lower than those typically reported for soils from  
720 other environments (tropical, temperate, Arctic) and are likely influenced by the low pH of  
721 the peat environment.

722 BHP signatures in peat are unique and do appear to record specific peat  
723 biogeochemical and ecological features, albeit with complex controls which are not yet  
724 fully understood. They also have strong preservation potential such that they could be  
725 useful in examining peat paleo-archives.

726

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739

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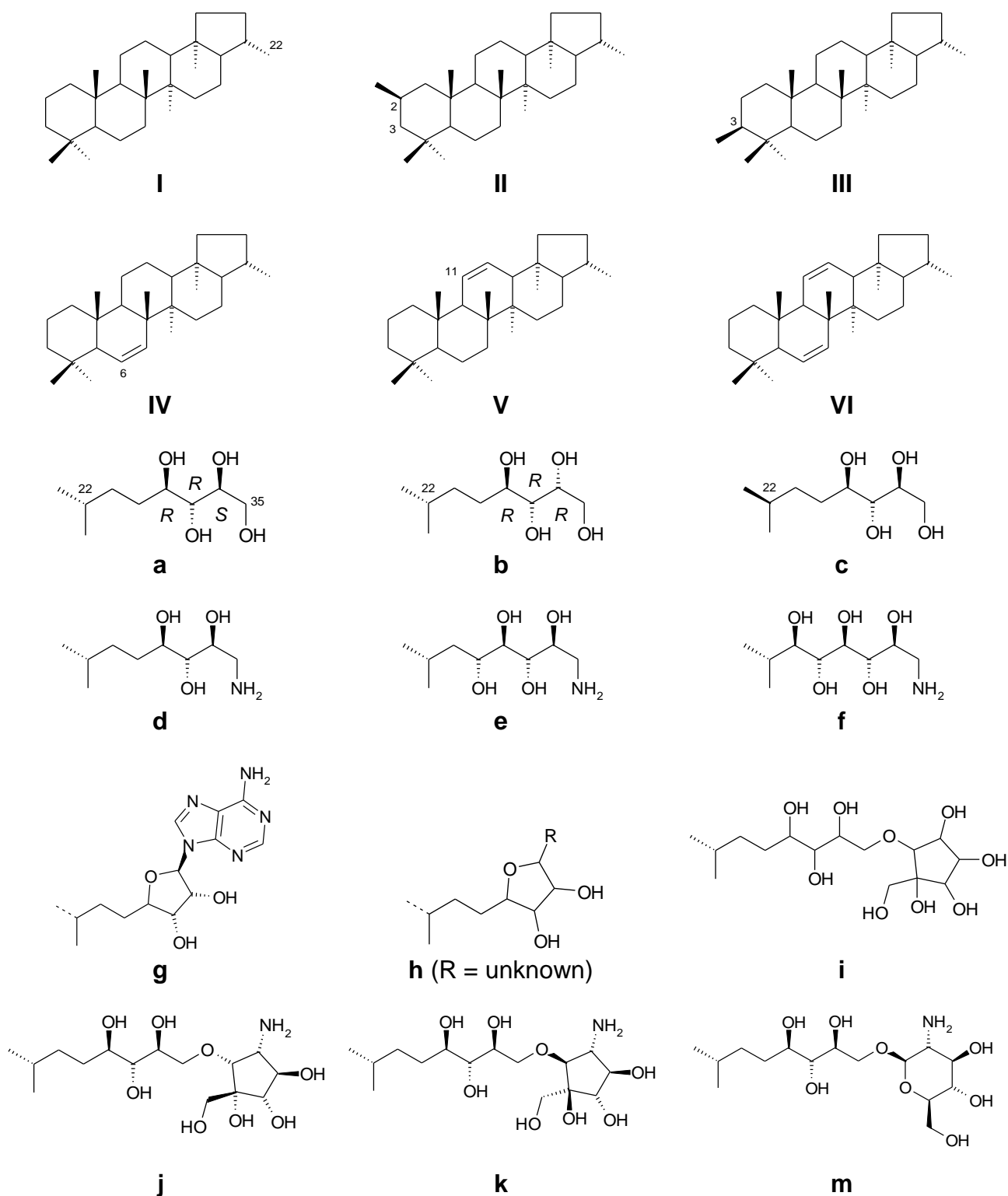
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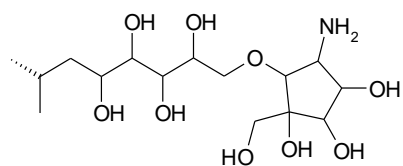
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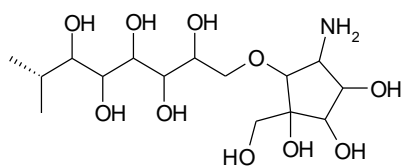
1165 **Appendix 1.**

1166 Ring system and side chains of BHPs observed in peat samples. Side chain structures h, i,  
 1167 n and o are based on LC-MS<sup>n</sup> analysis only. All other side chain structures shown have  
 1168 previously been unambiguously identified by NMR. When identified in this study using LC-  
 1169 MS only where stereochemistry can not be confirmed, we have assumed the structure to  
 1170 be the same as that previously characterised but the occurrence of additional/alternative  
 1171 isomers of the side chain cannot be excluded.  
 1172

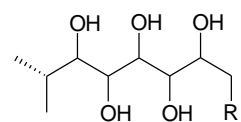




**n**



**o**



**p** (R= unknown)

1173

1174

1175

## 1176 List of Figures

1177

1178

1179 **Figure 1.** Concentration (black bars;  $\mu\text{g g}^{-1}\text{TOC}$ ) and relative abundance (open diamonds)  
1180 as % of total BHPs of dominant BHPs (a) BHT, (b) BHT-Cyclitol ether, (c) aminotriol, and  
1181 methanotroph specific markers (d) aminotetrol and (e) aminopentol. Grey shaded area  
1182 indicates region of water table variability (0-56 cm; Charman et al., 2007).

1183

1184 **Figure 2.** Concentration (black bars,  $\mu\text{g g}^{-1}\text{TOC}$ ) and relative abundance (open diamonds)  
1185 as % of total BHPs of (a) combined unsaturated BHT [2 isomers], (b) unsaturated BHT-  
1186 pentose, (c) BHT-pentose, (d) adenosylhopane (e) combined total 2-  
1187 methyladenosylhopane, Adenosylhopane-group 2 and C-2 methylated adenosylhopane-  
1188 group 2 (see appendix for individual structures). Grey shaded area indicates region of  
1189 water table variability (0-56 cm; Charman et al., 2007).

1190

1191 **Figure 3.** Relative abundance of soil marker BHPs with depth in *Sphagnum* peat samples  
1192 as % of total BHPs (a). Frequency of samples with relative abundance of soil marker BHPs  
1193 in terrestrial samples in indicated ranges from (b) Peat, (c) Tropical and sub-tropical soils  
1194 (d) Temperate soils and (e) Arctic samples (Key: OM and S = Organic matter and surface  
1195 permafrost soil; D = deep permafrost soils; IC = Ice complex; c = polygon centre; r =  
1196 polygon rim). (Data from this study; Cooke et al., 2008a; Xu et al., 2009; Rethemeyer et  
1197 al., 2010; Kim et al., 2011; Zhu et al., 2011; van Winden et al., 2012a,b; Wagner et al.,  
1198 2014; Doğrul Selver et al., 2015; Höfle et al., 2015; Spencer-Jones et al., 2015).

1199

**Table 1.** Intact bacteriohopanepolyols identified in BM peat sample and potential sources known to be found in peat including heterotrophs, methanotrophs and phototrophs. (Note some species also produce other compounds not observed in this study; See Talbot et al., 2008 for review.)

45

**Table 1. Continued**

APCI Base Peak ion ( <i>m/z</i> ) BHP structure number <sup>b</sup>	746 <sup>a</sup> lg	760 <sup>a</sup> lig	761 lh	775 llh	712 IVd or Vd	714 ld	728 lld	728 llld	772 le	830 lf	653 IVa,b or c	655 la	669 lla	1002 lj and/or lk	1002 lm	1058 IVn or Vn	1060 ln	1118 lo	1132 lp	941 IVi or Vi	943 li	957 lli	References
<u>METHANOTROPHS</u>																							
Alphaproteobacteria																							
<i>Methylocella</i> spp.	+					+						+											van Winden et al., 2012a
<i>Methylosinus</i> sp.						+			+			+											Neunlist and Rohmer, 1985b
<i>Methylocystis</i> sp.						+			+														Talbot et al., 2001
Gammaproteobacteria																							
<i>Methylovulum</i> sp. <sup>e</sup>					+	+			+	+													van Winden et al., 2012a
<i>Methylomonas</i> sp.									+	+													Neunlist and Rohmer, 1985a
<i>Methylococcus</i> sp. <sup>e</sup>									+	+													Neunlist and Rohmer, 1985a
<i>Methylocaldum</i> spp. <sup>e</sup>									(+)	+													Cvejic et al., 2000a
Verucomicrobia						+																	van Winden et al., 2012a
<u>PHOTOTROPHS</u>																							
Alphaproteobacteria																							
PNSB <sup>i,e</sup>	+		(+)		(+)	(+)						(+)	(+)	(+)	(+)								Talbot et al., 2007a
<i>Rhodoblastus</i> sp.	+											+		+	+								Talbot et al., 2007a
Cyanobacteria <sup>e</sup>	(+)					(+)	(+)				(+)	(+)	(+)	(+)	(+)		(+)			(+)	(+)	(+)	Talbot et al., 2008
<i>Gloeocapsa</i> sp.											+	+	+							+	+	+	Talbot et al., 2008

<sup>a</sup> Di, tri- and tetraacetate forms are known and observed here.

<sup>b</sup> See Appendix for structures

<sup>c</sup> BHP producing organisms are listed by group (Heterotroph, Methanotroph, Phototroph) and within group by Phylum. Specific genera or species are listed only if considered to potentially be present in *Sphagnum* peat (see text for further details); alternatively only BHP biosynthesised by other members of the phylum/sub-group are indicated

<sup>d</sup> AAB = acetic acid bacteria

<sup>e</sup> indicates organism also produces other BHPs not identified in this study

<sup>f</sup> + = present in species (or all members of group tested to date; see Talbot et al., 2008 for review)

<sup>g</sup> number of species identified to produce BHPs

<sup>h</sup> (+) indicates compound found in some but not all tested members of group/genus

1214    <sup>i</sup> PNSB = Purple non-sulphur bacteria.  
1215



1216

1217 Table 2. Total organic Carbon (%) and concentration of individual BHPs ( $\mu\text{g g}^{-1}\text{TOC}$ ) and grouped structural types in peat samples from

1218 Bissendorfer Moor.

Base peak (m/z)		653	653	655	669	712	714	728	728	772	830	746	760	761	775	941	943	957
Depth	% TOC	IVa,	IVa,	la	lla	IVd or	lb	llb	lllb	le	lf	lg	llg	lh	lh	IVi or	li	lli
cm		b or c	b or c			Vd										Vi		
0-2	41.440	21	18	20	6.4	0.00	69	1.4	bdl <sup>a</sup>	1.3	bdl	9.2	bdl	bdl	1.0	160	36	bdl
2-4	40.155	35	40	280	75	15	860	10	bdl	8.9	bdl	250	15	40	24	190	150	bdl
4-6	43.335	18	33	47	12	12	240	bdl	bdl	bdl	bdl	24	bdl	3.3	bdl	290	95	bdl
6-8	40.885	11	8.0	58	19	4.9	280	bdl	bdl	2.0	bdl	30	bdl	7.8	7.2	63	48	bdl
8-10	42.030	10	15	90	28	4.8	240	5.0	bdl	1.8	bdl	98	5.3	13	7.3	48	47	bdl
10-12	44.910	3.3	bdl	46	18	4.1	140	bdl	bdl	1.0	bdl	62	bdl	6.0	4.2	23	30	bdl
12-14	44.705	bdl	bdl	41	14	bdl	200	bdl	bdl	1.8	bdl	36	bdl	7.9	1.3	13	15	bdl
14-16	45.495	0.8	bdl	14	12	1.1	79	2.1	bdl	1.4	bdl	12	0.2	bdl	bdl	2.7	4	bdl
16-18	42.385	bdl	bdl	110	68	bdl	380	6.5	bdl	8.0	bdl	47	bdl	bdl	bdl	23	43	bdl
18-20	41.620	bdl	2.4	52	24	1.3	170	1.8	1.1	2.2	bdl	23	bdl	bdl	bdl	7.0	13	bdl
20-22	42.095	bdl	bdl	120	42	bdl	470	16	4.4	13	bdl	45	4.2	bdl	bdl	20	50	bdl
22-24	44.305	bdl	bdl	120	31	2.0	300	11	2.9	5.9	0.5	35	1.1	bdl	bdl	10	29	bdl
24-26	41.900	bdl	bdl	180	82	bdl	390	21	7.5	22	3.5	69	bdl	bdl	bdl	bdl	49	bdl
26-28	42.450	bdl	bdl	400	150	bdl	680	46	bdl	37	3.9	210	bdl	bdl	bdl	bdl	82	bdl
28-30	42.985	bdl	bdl	190	90	bdl	400	27	bdl	16	1.4	66	2.2	bdl	bdl	bdl	35	bdl
30-32	43.340	bdl	bdl	77	29	bdl	98	7.3	bdl	7.5	bdl	32	0.7	bdl	bdl	bdl	10	bdl
32-34	43.290	bdl	bdl	27	9.5	bdl	51	2.5	bdl	2.8	bdl	9.0	0.1	bdl	bdl	bdl	7	bdl
36-38	44.485	bdl	bdl	93	28	bdl	210	15	1.9	9.4	bdl	36	bdl	bdl	bdl	bdl	50	bdl
40-42	45.460	bdl	bdl	84	17	bdl	160	7.7	bdl	4.9	1.2	31	0.5	bdl	bdl	bdl	17	bdl
70-72	43.100	bdl	bdl	26	4.3	bdl	140	6.5	bdl	3.9	0.9	13	bdl	bdl	bdl	bdl	8	bdl
96-98	44.100	bdl	bdl	33	5.8	1.1	160	7.2	1.2	4.2	1.5	12	0.3	bdl	bdl	1.9	6	bdl
100-102	44.775	bdl	bdl	66	5.7	bdl	300	9.4	bdl	7.7	1.9	12	bdl	bdl	bdl	bdl	16	bdl
126-128	42.890	bdl	bdl	63	7.5	1.9	320	26	bdl	5.0	2.6	24	bdl	bdl	bdl	2.8	7	bdl
402-404	46.755	13	10	370	79	3.5	910	190	8.6	19	4.3	92	bdl	4.8	bdl	42	230	5.00
404-406	47.720	13	7.6	420	100	3.2	600	91	9.6	17	3.4	130	3.6	7.4	bdl	46	190	11.00
406-408	49.485	6.0	5.1	210	38	2.7	310	30	2.5	10	1.9	36	bdl	1.5	bdl	16	63	4.20

1219

408-410 48.515 4.7 6.1 170 27 1.5 440 33 2.7 8.7 2.0 32 bdl 2.0 bdl 15 60 0.00

1220 Table 2. Continued

Base Peak (m/z) Depth cm	1002 Ij and/ or Ik	1002 Im	1058 IVn or Vn	1060 In	1118 Io	1132 Ip	Total $\mu\text{g g}^{-1}\text{TOC}$	% Tetra <sup>b</sup>	% Penta <sup>b</sup>	% Hexa <sup>b</sup>	% Soil <sup>b</sup>	% Unsat <sup>b</sup>	R <sub>soil</sub> <sup>c</sup>
0-2	50	3.2	3.9	8.9	14	15	438.3	87.8	3.2	6.6	2.3	46.3	0.34
2-4	560	28	4.8	45	30	44	2704.7	82.9	2.2	2.7	12.2	10.5	0.54
4-6	180	14	7.2	26	37	18	1056.5	89.1	3.1	5.2	2.6	34.1	0.37
6-8	110	12	1.2	6.0	12	4.2	684.3	89.7	1.3	2.4	6.6	12.9	0.44
8-10	110	9.0	1.9	11	12	4.3	761.4	79.7	1.9	2.1	16.2	10.5	0.58
10-12	100	7.7	bdl	4.3	3.6	3.7	456.9	81.4	1.2	1.6	15.8	6.7	0.61
12-14	91	2.1	bdl	6.6	4.8	1.4	435.9	86.3	1.9	1.4	10.4	3.0	0.52
14-16	34	0.9	bdl	1.2	0.7	0.0	165.9	90.7	1.6	0.4	7.4	2.8	0.47
16-18	180	8.7	bdl	7.4	7.9	2.5	892.0	91.8	1.7	1.2	5.3	2.6	0.30
18-20	69	2.6	0.2	2.2	2.9	0.6	375.3	91.7	1.2	0.9	6.1	2.9	0.31
20-22	400	18	bdl	13	5.4	2.3	1223.3	93.2	2.1	0.6	4.0	1.6	0.29
22-24	210	4.5	bdl	9.0	4.0	1.2	777.1	92.7	1.9	0.7	4.6	1.5	0.23
24-26	440	15	bdl	19	12	1.4	1311.4	90.3	3.1	1.3	5.3	0.0	0.28
26-28	930	bdl	bdl	28	8.3	5.0	2580.2	88.7	2.5	0.7	8.1	0.0	0.34
28-30	300	12	bdl	2.6	4.5	1.8	1148.5	91.8	1.6	0.7	5.9	0.0	0.26
30-32	65	2.0	bdl	1.7	0.9	bdl	331.0	87.1	2.8	0.3	9.9	0.0	0.30
32-34	45	1.2	bdl	0.7	0.4	bdl	155.8	91.7	2.2	0.3	5.8	0.0	0.25
36-38	180	7.6	bdl	4.3	2.5	bdl	637.7	91.8	2.1	0.4	5.6	0.0	0.28
40-42	110	1.5	bdl	1.9	1.2	bdl	437.9	90.7	1.6	0.5	7.2	0.0	0.27
70-72	150	2.5	bdl	2.1	0.9	bdl	358.4	94.2	1.7	0.5	3.6	0.0	0.33
96-98	130	2.0	bdl	1.0	1.3	0.5	369.3	94.4	1.4	0.9	3.3	0.8	0.27
100-102	260	3.3	bdl	3.3	2.3	bdl	687.6	96.0	1.6	0.6	1.7	0.0	0.15
126-128	400	5.8	0.2	5.0	2.5	1.0	874.2	95.4	1.2	0.7	2.7	0.6	0.28
402-404	740	25	0.7	23	8.3	4.6	2782.8	94.4	1.5	0.6	3.5	2.5	0.21
404-406	630	7.3	0.5	26	11	7.0	2334.6	91.2	1.9	0.9	6.0	3.0	0.25
406-408	340	3.7	0.3	9.0	7.7	2.9	1100.5	93.7	1.8	1.1	3.4	2.7	0.15

408-410	290	3.6	0.5	6.8	5.2	2.8	1113.6	94.6	1.4	0.9	3.1	2.5	0.17
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1221 <sup>a</sup> bdl = below detection limit;

1222 <sup>b</sup> tetra = tetrafunctionalised BHPs with functional groups at C32, 33, 34 and 35, penta = pentafunctionalised with additional functional  
 1223 group at C31, hexa = hexafunctionalised with additional functional groups at C30 and 31, soil = all soil-marker BHPs inclidng  
 1224 adenosylhopane and related structures (**lg, llg, lh, llh**), unsat = the combined relative % of all unsaturated BHPs (tetra- and  
 1225 pentafunctionalised) with ring systems **IV** and/or **V**;

1226 <sup>c</sup>  $R_{soil} = (\mathbf{lg+llg+lh+llh})/(\mathbf{la+lg+llg+lh+llh})$  (Zhu et al., 2011).

1227

1228

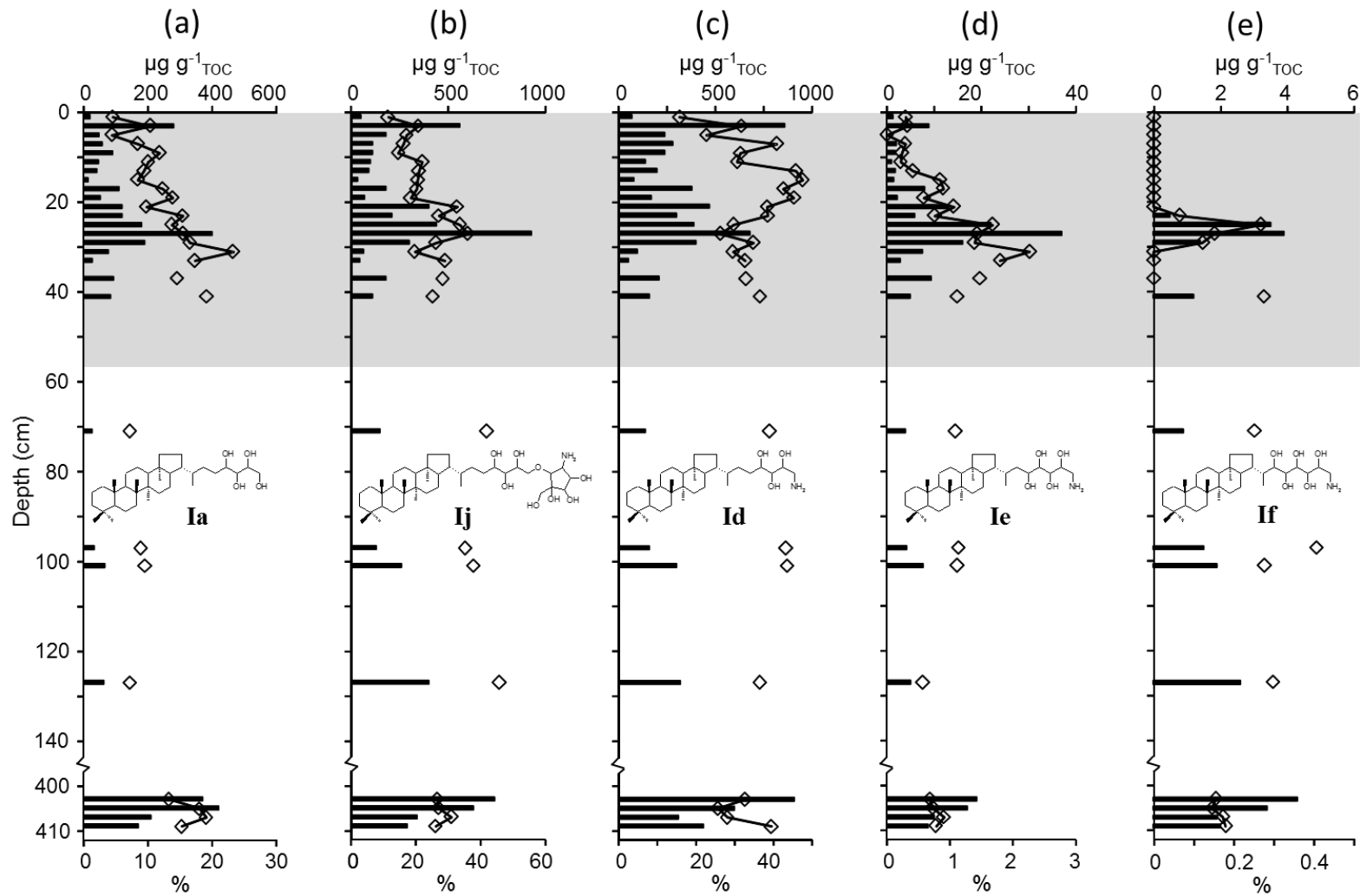


Figure 1

1229

1230

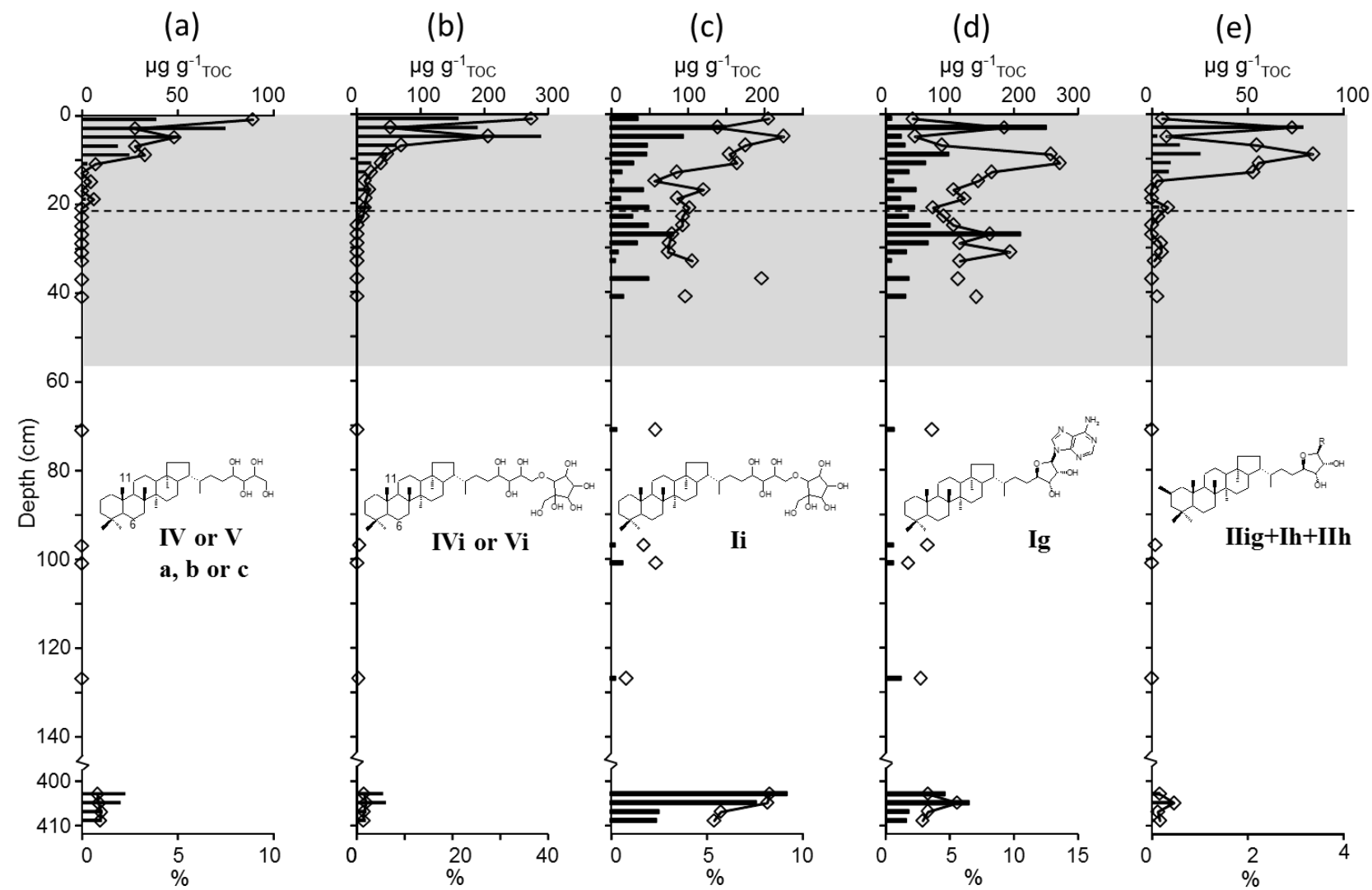


Figure 2

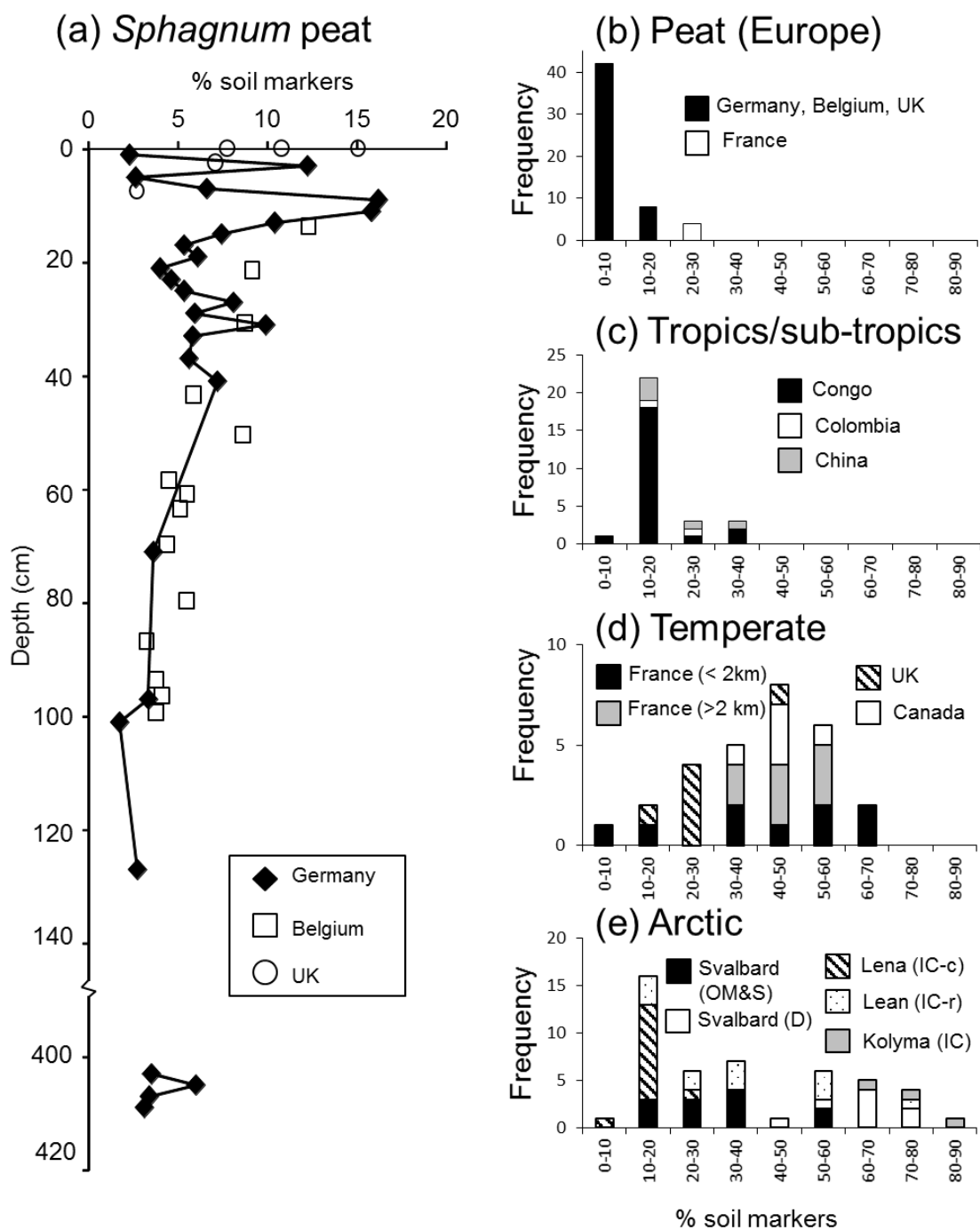


Figure 3